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RELATIONSHIP OF SERO-AGGLUTININ TITERS TO UDDER INFECTION IN STRAIN 19 VACCINATED CATTLE

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Since the initiation of vaccination of cattle with Brucella abortus strain 19 in 1940 as an adjunct to the State-Federal programs of brucellosis control and eradication in this country, the need for information that will make possible the correct determination of the brucellosis status of vaccinated animals with low agglutinin titers has increased with the vast number of animals vaccinated.

With the decline of bovine brucellosis from 6.8 percent in the fiscal year 1936 to 2.6 percent in the fiscal year 1954, as determined by the blood serum agglutination test, the number of vaccinated and nonvaccinated animals with low or fluctuating blood serum agglutinin titers has taken on a new significance. It is this group of animals that confounds the herd brucellosis status throughout the country with regards to conforming with present and proposed health department milk ordinances and interstate regulations for shipment of breeding cattle.

This experiment was designed to develop information on the relationship between blood serum agglutinin titers and udder infection in calf-vaccinated cattle.

Since Schroeder and Cotton[5] in 1912 reported the isolation of Brucella abortus from milk by means of guinea pig inoculation, most investigators of brucellosis have to some degree made direct culture and guinea pig inoculation studies along these lines. It is impractical to discuss the large number of publications on this subject at this time. Although studies of direct culture and guinea pig inoculation of milk have been conducted to attain

information relative to many different problems concerning brucellosis, most reports deal with nonvaccinated animals. The results of these studies are very similar when the status and source of the animals and conditions of the experiments are considered.

It was felt that direct culture and inoculation of guinea pigs with quarter samples of milk from the cows studied in this experiment would give us a fairly reliable and consistant means of identifying infected animals that were excreting Brucella from the udder. This is based on an isolation rate of 100 percent from the milk (quarter samples) collected under ideal conditions at weekly intervals from two groups of infected cows. Milk was collected for four weeks from four cows and for seven weeks from three cows. We appreciate that when a single milk collection is used to identify infected animals, 100 percent accuracy is seldom attained because of unfavorable conditions frequently encountered in the field. Regardless of this it would not alter the trend of relationship of isolations to blood serum titers. Furthermore, we believe that the suggested procedure is the most practical method that can be applied in a study of this kind.

EXPERIMENTAL PROCEDURES

Blood and milk from 740 cows of all dairy breeds as well as the cross-breeds usually found in milk producing areas of this country were examined. These cows were from 278 herds containing 6,276 adult cows. In each herd selected, all animals showing a significant reaction in the 1:50 or higher dilution of the standard sero-agglutination tests for brucellosis were studied. Results of the last test conducted by the State-Federal disease control organization, which in most cases was approximately

30 days prior to our sample collections, were used in selecting herds and cows for this study.

In the first part of the experiment, studies were conducted on 263 cows from 180 herds in Jersey County, Illinois, This county had been participating in an intensive calf-vaccination program since 1942, or approximately ten years prior to this study. According to information furnished by Dr. A. K. Kuttler's office, the cows in this county were blood tested only at the beginning of the program in 1942 and at intervals of five years thereafter to evaluate progress in control of brucellosis. Animals were eliminated only by natural cause and when they became unprofitable.

After completion of this part of the experiment it was thought advisable to also study cattle from an area where the herds were larger, the percentage of infection was higher, and where there was a variation in brucellosis control measures, i.e. test and slaughter, calf-vaccination, or a combination of both and no control efforts in some cases. New York State was selected as the area to conduct the second part of the experiment. Examinations were made on 477 cows in 98 herds.

All of the cattle from both areas were from herds containing reactors or suspects with 70.3 percent of the vaccinated and 70.8 percent of the nonvaccinated ones from herds harboring reactors. The average age at time of vaccination was 8.7 months.

Quarter samples of udder secretion were collected aseptically from cattle of various ages (2 to 12 years), in all stages of lactation, and in some cases from nonlactating cattle. The amount of secretion collected was 30 ml. when available. The udders were washed with a solution of

quatinary ammonium compound, rinsed with clear water and wiped dry. The teats were then cleansed with a pledget of cotton soaked with 70 percent alcohol; special attention being given to the orifices of the teat canal. Two or three streams of milk were discarded before collection of the sample. A 30 ml. sample of blood was drawn from the jugular vein at the time of milk collections. Milk and blood samples were placed under refrigeration and shipped by air express to the Animal Disease Station within 24 hours after collection.

The blood samples were centrifuged and serum was separated from the clot. A portion of each serum sample was subjected to the standard tube and plate agglutination tests for brucellosis. The remainder of each serum sample was stored for future studies on specific and nonspecific agglutination reactions.

Milk samples were allowed to stand at refrigerator temperature over night so the cream could rise. Petri dishes containing serum potato agar medium were inoculated with 0.2 ml. of cream from each sample. The inoculated medium was then incubated at 37.5°C. in an atmosphere of 10 percent carbon-dioxide for seven days at which time observations were made for the presence of Brucella colonies.

Immediately following inoculation of the medium, the remaining cream and sufficient quantity of milk and sediment from each sample to make up 5 ml. was injected intraperitoneally into guinea pigs. Each quarter sample collected in Illinois was inoculated into two guinea pigs, whereas each quarter sample collected in New York was inoculated into one guinea pig.

When sufficient quantities were available, a portion of each quarter sample was treated with rennin for subsequent tests to determine the presence

of Brucella agglutinins in the whey. The milk ring test was conducted on composite quarter samples of milk from each cow tested in Illinois and on can samples of milk from each herd tested in New York. The milk from Illinois was tested in our laboratory and that from New York in the State laboratory at Albany.

All guinea pigs inoculated with udder secretion from each cow were isolated in a separate cage for approximately 35 days. They were then sacrificed, autopsied, and observed for any gross lesions indicative of brucellosis. A blood sample was taken from each pig and tested for Brucella agglutinins. The spleen of each pig was removed and cultured as were other organs or lymph nodes suspected of localized infection. These tissues were cultured directly on serum potato agar slants and incubated in the same manner as that employed for direct culture of cream.

Suspicious colonies observed during any of the culturing procedures were subcultured so that they could be examined for staining characteristics, motility, antigenicity, CO_2 requirement, and cellular and colonial morphology. All isolations of Brucella were further examined for dye inhibition, urease activity, hydrogen sulphide production, and antigenic activity with mono-specific sera in order to classify them according to specie.

Every effort was made to obtain a complete history of each herd and animal. This information included number of adult animals, previous blood tests, method of adding or replacing animals, number of years that calf-vaccination had been practiced, number of abortions, vaccination and present age of each animal and stage of lactation. Any animal whose vaccination status was not clearly established was omitted when results were compiled.

RESULTS

Isolations of Brucella were made from the milk of cows in all stages of lactation and in some cases from the scant secretions of nonlactating animals. The types of udder infection differed in that some were apparently disseminating Brucella from only one quarter, whereas others from two or more quarters.

All isolations were virulent strains of Brucella abortus. Thirteen of the 51 isolations were atypical in that they were inhibited by routine and in most cases by lesser concentrations of basic fuchsin and methyl violet used for typing Brucella. Their reactions to other typing procedures and mono-specific sera were typical of virulent Brucella abortus. Twelve of these atypical Brucella abortus isolations were obtained from cows in New York and one from a cow in Illinois. The organisms were similar to those first described by Wilson[6], and more recently reported by Huddleson[3,4]. However, they were not associated with mastitis at the time of isolation.

The results of studies on relationship of blood serum agglutinin titers to isolations of Brucella abortus from milk of calf-vaccinated and nonvaccinated cows are shown in Table I. Although it was not our intention to conduct studies on animals with sero-agglutinin titers lower than I 1:50, there were some vaccinated and nonvaccinated animals whose titers had receded below this level since the pre-milk-collection test. No isolations of Brucella were made from the milk of vaccinated cattle with titers less than I 1:100 nor from nonvaccinated cattle with titers less than + 1:50. Of animals classified as suspects (titers of I 1:50 to I 1:100 inclusive) Brucella was isolated from 0.51 percent of the vaccinated and 2.7 percent of the nonvaccinated cattle. Furthermore, udder infection was demonstrated in 15.34 percent of the vaccinated reactors and 48.93 percent of the nonvaccinated

reactors. However, in both classes of animals with titers of I 1:400 or higher, the percentages of demonstrable udder infection were almost equal. The percentage of infection in vaccinated cattle at the various titer levels was approximately the same in all age groups. To summarize, Brucella was isolated from the milk of 29 of637 vaccinated cattle or 4.55 percent as compared to 22 of 103 nonvaccinated cattle or 21.35 percent.

Efforts to determine changes in titers of suspects and low-titer reactors subsequent to isolation of Brucella were unsuccessful because in each case these animals had been removed from the herd.

Figure 1 shows more graphically the relative trend of Brucella isolations to the sero-agglutinin titers of both vaccinated and nonvaccinated cattle. No isolations of Brucella were made from the milk of vaccinated cows with titers of I 1:200 or from nonvaccinated cows with titers of I 1:100. However, since Brucella was isolated from cows with titers at the preceeding and suceeding levels, it is logical to assume that udder infection could have been demonstrated if adequate numbers had been available. Therefore, the probable percentages of Brucella isolations that may be expected were calculated by applying the formula for geometric progression. These calculated percentages are almost identical. The percentage of infection was approximately two to four times greater in the nonvaccinated than in the vaccinated group at each titer level shown except for those animals with titers of I 1:400 or higher. The most precipitous rise in percentage of infection began with the + 1:100 titer in nonvaccinated and + 1:200 titer in vaccinated cattle.

Although the number of nonvaccinated cattle examined was limited, our results compare favorably with those reported by Everson et al[1] who examined blood and udder secretions of 714 cows, and where the majority of conditions were similar.

An extremely significant finding is that all but 1 of 51 Brucella-positive cows were from herds which contained one or more animals with a maximum sero-agglutinin titer of I 1:400 or higher. The one exception was a ten-year old nonvaccinated cow with a titer of + 1:50 and was in a herd of 30 cows where the maximum individual sero-agglutinin titer was I 1:100. The origin of this animal is unknown; however, the history of the herd shows that there was no record of a previous agglutination test, calfhood vaccination had been practiced for the last 8 years and at least 50 percent of the cattle replacements were purchased. Another important observation is that 73.07 percent of the 26 herds containing bacteriologically positive cattle were supplemented by purchased replacements.

The results of the milk ring test on can samples from 96 of the 98 herds studied in New York are presented in Table II. All except 1 of the 17 herds containing bacteriologically positive cows had milk reactions of 2+ or higher. This one exception contained 58 adult cattle; six of which had titers no higher than + 1:100 whereas the remaining one had a titer of + 1:1600 and was bacteriologically positive. Failure of the milk ring test to identify this infected herd is difficult to explain since all of the quarter milk samples from the infected cow had whey titers of + 1:100 or 1:200. Sixty-two of the 79 bacteriologically negative herds sampled were negative to the ring test; whereas, 12 showed reactions of 2+ or higher and 5 showed reactions of 1+. Summarily, the ring test conducted on herds practicing calf-vaccination correctly identified 94.12 percent of those that were bacteriologically positive and 78.5 percent of those that were bacteriologically negative.

The efficiency of the milk ring test for properly identifying herds that have been classified by the standard sero-agglutination test was also evaluated. In the herds containing cattle with maximum titers of I 1:100 or less, 92.31 percent were classified as brucellosis-free by the milk ring test; however,

it also classified 37 of the 48 or 77.08 percent of the herds with maximum titers of + 1:100 to + 1:200 inclusive as negative. Therefore, the milk ring test results were in closer agreement with the bacteriological findings than the serological findings in calf-vaccinated herds with maximum titers of + 1:100 to + 1:200. Nevertheless, this test identified 90.09 percent of the herds with maximum sero-agglutinin titers of I 1:400 or higher as infected which also compares favorably with the bacteriological findings.

The milk ring test was not conducted on a herd basis in Jersey County, but composite quarter samples of each cow were examined. In each case where Brucella isolations were made, the milk was positive to the ring test. Results of the ring test on individual cows have been disregarded because of the high percentage of positive reactions in the milk of bacteriologically and serologically negative animals. The factors responsible for the inconsistant results were acidification of the milk, mastitis and early and late stages of lactation.

The results of studies conducted to show the relationship of milk whey agglutinin titers to blood serum agglutinin titers and isolations of Brucella from the milk of both vaccinated and nonvaccinated cows are shown on Table III. If whey agglutinin titers of 1:25 or higher are considered indicative of infection, the whey agglutination test correctly identified 39 or 81.25 percent of 48 bacteriologically positive cattle and 548 or 84.18 percent of 651 bacteriologically negative cattle tested. In comparison, since blood serum agglutinin titers of + 1:100 or higher are accepted as the established criterion of infection, 45 or 93.75 percent of the same 48 cattle were identified correctly by the sero-agglutination test. Both tests were in agreement on classifying 38 of 48 bacteriologically positive cows; however they failed to properly identify one. Of the 10 infected animals on which these test results

disagreed, the whey test correctly identified two cows that were classified as uninfected by the sero-agglutination test but failed to identify eight that were properly classified by that test.

DISCUSSION

The significance of using udder infection as a criterion of brucellosis in cows is demonstrated by our findings that Brucella abortus was isolated from the udders and supramammary lymph glands of 93.5 percent of 92 infected cows at the time of autopsy. Regardless of procedures employed for controlling brucellosis, none are absolutely perfect. This is also true with diagnostic tests, regardless of the titer level used for indicating infection. With any diagnostic level, there is always the possibility of not identifying some infected animals, however, this calculated risk should be similar in both nonvaccinated and calf-vaccinated cattle regardless of the levels selected.

The data presented strongly suggests that serious consideration must be given to liberalizing the interpretation of the sero-agglutination test on properly vaccinated cattle if discrimination against these animals is to be eliminated and calf-vaccination is to remain an integral part of the brucellosis control and eradication program. In addition, more emphasis must be placed on the brucellosis status of the herd if sound judgment is to be exercised in disposing of individuals with questionable titers and in recommending scund control procedure. All of the results of our studies fully support this philosophy, and is emphatically demonstrated by the fact that 98.04 percent of the virulent Brucella abortus isolations were from cattle in herds which harboured one or more animals with sero-agglutinin titers of I 1:400 or higher.

The percentage of demonstrable udder infection was two to four times greater in nonvaccinated than in vaccinated cattle in all titer levels between

I 1:50 and I 1:400. If it is logical to accept the + 1:100 titer as the diagnostic level of infection in nonvaccinated cattle, it is just as logical to accept the + 1:200 titer as the diagnostic level of infection in officially calf-vaccinated cattle since these are the points where the infection rates rise sharply. This would mean that the diagnostic titer would be one dilution higher in vaccinated than in nonvaccinated cattle.

A comparison of present and alternate interpretations of the sero-agglutination test is presented in Table IV.

The infection rates in the three classifications of calf-vaccinated animals (reactor, suspect, negative) as determined by the alternate interpretation of the sero-agglutination test were almost identical to those in the same classifications of nonvaccinated animals as determined by the present interpretation. Consequently the risk of retaining infected cattle in herds is no greater in the calf-vaccinated animals than in the nonvaccinated animals. The significance of this is substantiated by the fact that the percentages of calf-vaccinated and nonvaccinated cattle which originated in infected herds were practically the same. In addition, use of the alternate interpretation of the sero-agglutination test to classify vaccinated cattle, reduced the number of reactors and suspects approximately 300 percent and 40 percent respectively and increased the number of negative animals approximately 500 percent.

The greatest benefits derived from use of the alternate interpretation of the sero-agglutination test will be realized only when calves are vaccinated at the proper age, i.e. 6 to 8 months. This can best be demonstrated by presenting unpublished data on the recedence of vaccinal titers in 88 heifers vaccinated at eight months of age and 73 heifers vaccinated at 12 to 15 months of age. Vaccinal titers of 95.46 percent of the animals vaccinated at eight

months of age had receded below the I 1:100 level by the time they had reached 30 months of age; whereas, the titers of only 43.83 percent of the animals vaccinated as yearlings had receded below the same level at the same age. Similar information on the relationship of vaccination age to recedence of titers was presented by Haring and Traum[2] in 1942. Consequently, any effort to use the alternate interpretation as an excuse to raise the vaccination age of heifers will nullify the potential advantages of this interpretation.

The role of calf-vaccination as an aid in controlling brucellosis can best be demonstrated in Jersey County, Illinois. After completion of an informative blood serum agglutination test on cattle in this area calf-vaccination was initiated on a rather large scale in 1942 and continued for at least the next 10 years. Elimination of infected cattle was done principally by disposing of them when they became unprofitable. Two subsequent, informative blood tests were conducted in the county at five year intervals. Reduction of the infection rate was discouraging at the end of the first five-year period but was rather spectacular at the end of the second five-year period. This is best demonstrated by giving the percentage of reactors found on the following tests: 5.018 percent in 1942, 3.7 percent in 1947 and 0,53 percent in 1952. In New York where 12 of the 13 atypical strains of Brucella abortus were isolated, there was no evidence that strain 19 vaccine was less effective against these variants than against typical strains of Brucella abortus. In the great majority of vaccinated herds where infection was found, there were histories of purchasing replacement animals. Even in these herds the disease was benign in character.

The relatively low sero-agglutinin titers found in adult cattle properly vaccinated as calves with strain 19 does not appear to materially affect the efficiency of the milk ring test as it is now applied in the field. Therefore, the test remains an effective diagnostic aid in the control of bovine

brucellosis. This was ably demonstrated by the findings that it correctly classified 94.12 percent of the bacteriologically positive and 78.5 percent of the bacteriologically negative herds. Furthermore, the milk ring test also classified the most potentially dangerous herds, which contain cattle with titers of I 1:400 or higher, as infected.

The whey agglutination test is also of value in diagnosing udder infection in the individual animal but is difficult to apply in the field. In addition, conditions such as mastitis and stages of lactation interfere considerably with the accuracy of the test on quarter milk samples. When comparative tests were conducted, the percentage of accuracy in correctly classifying cattle was greater with the sero-agglutination than with the whey agglutination test.

SUMMARY

Milk and blood samples were collected from 637 vaccinated and 103 nonvaccinated cattle for bacteriological and serological studies. The samples were obtained from 477 cows in New York State and 263 cows in Jersey County, Illinois.

Udder infection was demonstrated in 51 of the cattle studied. Thirty-eight of the Brucella abortus isolations were typical in all respects. Of the 13 atypical strains, 12 were isolated from cattle in New York State.

Brucella was isolated from the milk of 4.55 percent of the calf-vaccinated cattle and from 21.35 percent of the nonvaccinated cattle. The percentage of infection in vaccinated cattle at the various titer levels was approximately the same in all age groups.

The percentage of Brucella isolations was two to four times greater in the nonvaccinated than in the vaccinated cattle at each sero-agglutinin titer level except for those animals with titers of I 1:400 or higher where the

percentage was approximately the same. The most precipitous rise in percentage of infection began with the + 1:100 titer in nonvaccinated and + 1:200 titer in vaccinated cattle. Consequently, the diagnostic sero-agglutinin titer level was found to be one dilution higher for vaccinated than for nonvaccinated cattle if the present interpretation of this test is used as the standard.

Of all the isolations made from both vaccinated and nonvaccinated cattle, 98.04 percent were from cows within herds which contained one or more animals with sero-agglutinin titers of I 1:400 or higher.

No isolations of Brucella abortus were made from the milk of vaccinated cows with sero-agglutinin titers in dilutions below 1:100.

The milk ring test conducted on herds practicing vaccination correctly identified 94.12 percent of those that were bacteriologically positive and 78.5 percent of those that were bacteriologically negative.

When whey agglutinin titers of 1:25 or higher are considered indicative of infection, the whey agglutination test correctly identified 39 or 81.25 percent of 48 bacteriologically positive cattle and 548 or 84.18 percent of 651 bacteriologically negative cattle.

An alternate interpretation of the sero-agglutination test for brucellosis in classifying calf-vaccinated cattle has been discussed. The efficiency of this alternate interpretation in classifying calf-vaccinated cattle is nearly identical to that of the present interpretation in classifying nonvaccinated cattle.

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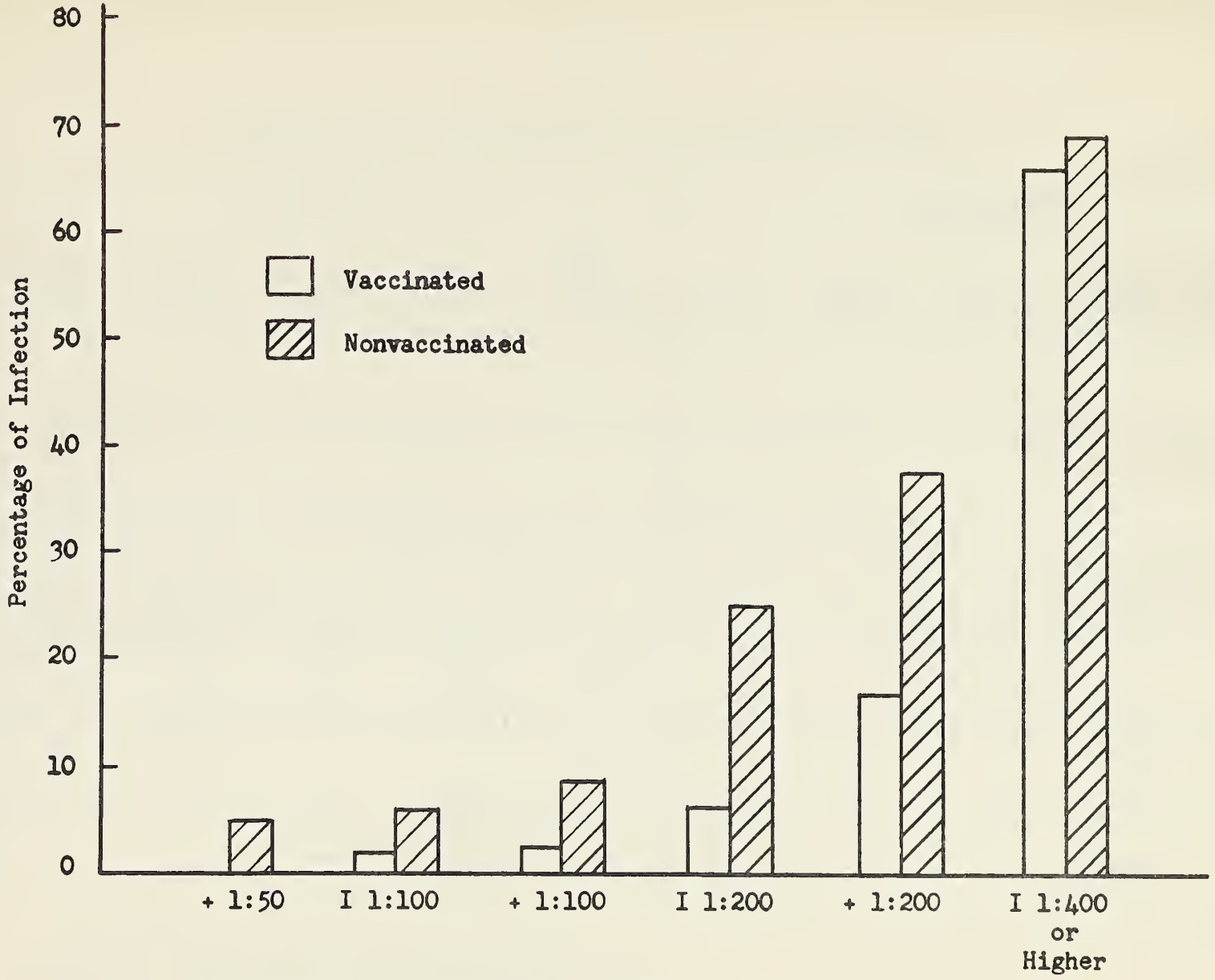

Figure 1. - Relationship of Sero-Agglutination Tests to Percentage of Infection

TABLE I

Relationship of Blood Serum Agglutinin Titers to Isolations of *Brucella abortus* from Milk of Vaccinated and Nonvaccinated Cows

Blood Serum Agglutinin Titers	Vaccinated					Nonvaccinated				
	No. of Cows	No. Br. Negative	No. Br. Positive	% Br. Positive	% Br. Positive Per Dil.	No. of Cows	No. Br. Negative	No. Br. Positive	% Br. Positive	% Br. Positive Per Dil.
- 1:25	6	6				5	5			
I 1:25	7	7				2	2			
+ 1:25	55	55				12	12			
I 1:50	48	48				7	7			
+ 1:50	236	236				24	23	1	4.16	3.22
I 1:100	109	107	2	1.82		6	6			
+ 1:100	94	92	2	2.12	1.97	12	11	1	8.33	5.55
I 1:200	31	31				4	3	1	25.0	
+ 1:200	18	15	3	16.66	6.12	8	5	3	37.50	33.33
I 1:400 or higher	33	11	22	66.66	66.66	23	7	16	69.56	69.56

- = no agglutination reaction in dilution shown
I = incomplete agglutination reaction in dilution shown
+ = complete agglutination reaction in dilution shown
Dil. = dilution

TABLE II

Correlation of Milk Ring and Sero-agglutination
Tests to Isolations of Brucella abortus from Milk

Maximum Milk Ring Reaction in Each Herd	Maximum Sero-Agglutinin Titer in Each Herd									
	Negative 1:25	I 1:25	+ 1:25	I 1:50	+ 1:50	I 1:100	+ 1:100	I 1:200	+ 1:200	I 1:400 or Higher
No Reaction				1	8	15	21	8	8	2 (1)
1+						1	1	1	1	1
2+						1 (1)	2	1	1	2 (1)
3+									2	5 (3)
4+							1	1		12 (11)

Figures in parenthesis represent number of herds from which Brucella was isolated

TABLE III

Relationship of Milk Whey Agglutinin Titers to Blood Serum Agglutinin Titers and Isolations of Brucella from Both Vaccinated and Nonvaccinated Cows

Blood Serum Agglutinin Titers	Whey Agglutinin Titers							Total Serum Titers	Total Isolations	% Isolations
	Not Tested	Negative	1:25	1:50	1:100	1:200	1:400 or Higher			
Negative	1	9	1					11		
I 1:25		8		1				9		
+ 1:25	4	55	3	4	1			67		
I 1:50	1	52	1	2		1		57		
+ 1:50	13	216 (1)	5	8	10	3	4	259	1	.386
I 1:100	7	84	3	4	8 (1)	5 (1)	1	112	2	1.79
+ 1:100	10 (1)	81 (2)	2	3	6	1	5	108	3	2.78
I 1:200	1	26 (1)	1		4	3	1	36	1	2.74
+ 1:200		16 (3)	1	3 (1)	2 (1)	3 (1)		25	6	24.0
I 1:400 or higher	4 (2)	10 (2)	3 (1)	3 (2)	11 (10)	12 (10)	13 (11)	56	38	67.86
Total Whey Titers		557	20	28	42	28	24			
Total Isolations		9	1	3	12	12	11			
% Isolations		1.62	5.0	10.71	28.57	42.85	45.83			

Figures in parenthesis = number of cows bacteriologically positive for Brucella
Negative = no agglutination in 1:25 dilution

TABLE IV

Interpretations of the Sero-Agglutination Reactions
in Calf-Vaccinated and Nonvaccinated Cattle

Interpretations	Calf-Vaccinated Cattle				Nonvaccinated Cattle			
	Animals		Brucella Isolations		Animals		Brucella Isolations	
	%	No.	%	No.	%	No.	%	No.
Present								
Reactor - (+ 1:100 or Higher)	27.63	176	15.34	27	45.63	47	48.93	21
Suspect - (I 1:50 - I 1:100)	61.69	393	.51	2	35.92	37	2.7	1
Negative - (+ 1:25 or Lower)	10.68	68		0	18.45	19		0
Alternate								
Reactor - (+ 1:200 or Higher)	8.01	51	49.02	25				
Suspect - (I 1:100 - I 1:200)	36.73	234	1.71	4				
Negative - (+ 1:50 or Lower)	55.26	352		0				